PME RESEARCH LABORATORY, APRIL 1980

PROJECT TITLE : BIOTECHNOLOGY

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1. ANALYSIS OF C. UTILIS NCYC 707 (1)

C. utilis NCYC has been grown on three different media:

1. Extract of stem pool (stem : water = 1 : 10) having NO_3^- as nitrogen source.
2. Nitrate broth (Merck) having NO_3^- as nitrogen source.
3. Complex medium containing yeast extract, peptone and dextrose, having NH_4^+ as nitrogen source.

All media contained the same amount of glucose (4 %) and the same amount of nitrogen (1130 ppm). The yeast produced in these media was centrifuged, washed twice with water, freeze-dryed and analyzed for their Carbon, Hydrogen, Oxygen, Nitrogen and Sulfur content (2).

growth medium	% C	% H	% N	% O	% S
1	45.66	6.47	9.53	31.89	0.32
2	47.23	6.69	9.40	33.50	0.41
3	47.80	6.70	9.40	33.94	0.37

The values indicate that there is no significant difference in the composition of Candida utilis grown on different nitrogen substrates and in different media. The values of nitrogen are, however, surprising, as total nitrogen determinations in our laboratory gave a 45 % higher value with yeasts grown on ammonia than with yeasts grown on nitrate (3).

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2. ACID CONSUMPTION (4)

Measurements of acid consumption in the NINO process continued. Phosphoric acid was replaced by lactic acid. A strong correlation was observed between the pH of the denitration trial and the consumption of lactic acid. Every liter of extract (standard RL stem blend extracted 1 : 10 with water), containing 5 g/l NO_3^- and 0.2 % KH_2PO_4 the following amount of lactic acid (90 %) was consumed:

pH of the denitration	consumption of lactic acid (g/l)
4	50.0
5.5	38.4
6.3	32.9
6.8	25.7
7.0	14.1
7.3	3.1

At pH 7.3 the system was no longer working optimally. After 12 hours a residual nitrate level of 250 ppm could be measured.

3. STRIP EXTRACT: BIOCONVERSION OF NITRATE

The difficulties encountered earlier with the denitration at burley strip extracts according to the NINO process are now fully understood. They are due to the fact that under extreme extraction conditions a decomposition of N-containing constituents of the tobacco leaf occured whereby large quantities of ammonium ion were formed. The high ammonium concentrations inhibited in a competitive way denitration. Values of NH_3-N were found that exceeded the values of NO_3-N by a factor of up to five.

In burley tobacco the NO_3-N content is usually higher than the NH_3-N content. In all analyzed samples available in the TLA data bank the mean for NO_3-N is 0.6 percent and the mean for NH_3-N is 0.4 percent. There is no correlation whatsoever between those two variables. At high nitrate levels, the NO_3-N always exceeds the NH_3-N values.

It has been experimentally shown that if burley strips are extracted at lower temperatures where no decomposition of nitrogenous material occurs, the NH_3-N values are lower than the NO_3-N values in the extract, and normal NINO bioconversion is possible.

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4. MISCELLANEOUS

- Assistance has been given to the pilot plant for the ongoing trials.

- Media for tissue cultures have been prepared.

REFERENCES

(1) C.M. Aegerter, Notebook 115, 45-46

(2) Analytische Laboratorien, A. Bernhardt, Analysenbericht vom 11.4.80

(3) M.F. Mangilli, Notebook 791205 p. 32/33

(4) J. Berney, Notebook 128, 46-48

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